The rejection of claims 1-6 and 11-30 under U.S.C. §103(a) as being unpatentable over the combination of Manz et al and De Los Reyes et al., in view of Mobarry et al and Walder or Gordon is respectfully traversed with respect to the amend claims.

Applicants have amended claim 1 according to the suggestion of the Examiner stated in the Office Action under the subtitle, "Response to Argument". Amended claim 1 now states that the process of the invention includes the in situ hybridization of whole cells followed by extraction and quantification of the specific probe to qualitatively and quantitatively analyze microorganisms in a sample. Accordingly, Applicants respectfully request that this rejection be withdrawn.

The rejection of claims 1-12, 16-18 and 24-29 under 35 U.S.C. §103(a) as being unpatentable over Mayrand (US 5,691,146) in view of Burton and Nuovo (US 5,538,871) and in further view of De Los Reyes and Mobarry is respectfully traversed with respect to the amended claims. As stated in paragraph nine of the Office Action, both Mayrand and Burton, the primary and secondary references in the rejection, respectfully, disclose a process of what is commonly referred to as *in situ* PCR amplification. *In situ* PCR is concerned with the amplification of DNA to detect DNA with high sensitivity. Also, the classical PCR technique does not allow for the quantification of DNA in the original sample because the amount of amplified DNA is not proportional to the initial target quantity. In other words, classical *in situ* PCR is strictly a qualitative technique.

Applicants' inventive process is completely different than the PCR process described in Mayrand and Burton. First, Applicants target RNA, not DNA. Second, the RNA in the sample is not amplified by a thermal enzyme process (PCR). Instead, the claimed process is directed to the extraction and quantification of the probe after *in situ* hybridization. As a result, the claimed process allows for the quantification of cells by detecting the amount of RNA hybridized with the specific probe. Accordingly, Applicants respectfully request that the rejection be withdrawn.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

In view of the above, each of the presently pending claims in this application is believed

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue.

Applicants believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 22-0185, under Order No. 21029-00196-US from which the undersigned is authorized to draw.

Dated:

Respectfully submitted,

By _____

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Version With Markings to Show Changes Made

In the specification:

Please insert --FIELD OF INVENTION-- on page 1, line 6 after title of invention.

Please insert -- BACKGROUND -- on page 1, line 11 after the first paragraph.

Please insert -- SUMMARY OF THE INVENTION -- on page 4, after the first paragraph.

Please insert -- DETAILED DESCRIPTION OF THE INVENTION -- on page 4, before the paragraph beginning on line 19.

Page 4, last paragraph continuing to Page 5, line 15 should read as follow:

As used herein, the term "microbiological population" (or "microbiological domain") means the set of microorganisms that a given probe is able to recognize by recognition of an RNA target sequence present in each member of said set. The approach is based on oligonucleotide hybridization probes complementary to RNA sequences that are diagnostic for selected phylogenetic groups which correspond, to varying degrees, to a target region typical of a type of a microorganism or a whole group of microorganisms. Any probes enabling said contacting step is appropriate for the implementation of the method according to the invention. The choice of the specific probe(s) is directly related to the analysis desired for said sample. Probes can *e.g.* be composed of oligonucleotide sequences that can distinguish between the primary kingdoms (eukaryotes, eubacteria, archaebacteria) and between closely related organisms (the group of Ammonia-oxidizing β-Proteobacteria, the genus *Nitrobacter* or Acinetobacter or the species *Fibrobacter intestinalis,* the species *Escherichia Coli*). Probes with finer phylogenetic resolution can be derived by using the existing collections of RNA sequences. Many examples of such RNA-targeted probes are described in the prior art such as patents or patent applications, scientific publications *e.g.* Los Reyes *et al.* 1997, Appli. Environ. Microbiol. Vol. 63 [n°3] No. 3 p.1107-1117 ; Mobarry *et al.* 1996, Appli. Environ. Microbiol. Vol. 62 [n°6] No. 6 p.2156-2162 ; Wagner *et al.* 1994, Appli. Environ. Microbiol. Vol. 60 [n°3] No. 3 p.792-800 ; Kane *et al.* Appli. Environ. Microbiol. Vol. 59 [n°3] No. 3 p.682-686. Other examples of such probes can also be designed by the person skilled in the art. Advantageously probes are those which target ribosomal RNA (rRNA). Examples of such advantageous probes include Nb1000 (SEQ ID [N°] No. 1) and Nso 1225 (SEQ ID [N°] No. 2).

Page 11, second paragraph should read as follows:

c) Hybridization step

A water bath is prepared at the hybridization temperature required by the probe being used (the temperature depends on the length and sequence of the probe). In the example reported here, the following probes were used:

Probe Nb 1000 specific to the *Nitrobacter* genus, with sequence SEQ ID [n°1] No. 1: 5' TGCGACCGGTCATGG 3'

Probe Nso 1225, specific to Ammonia-oxidizing β proteobacteria, with sequence SEQ ID [n°2] No. 2: 5' CGCCATTGTATTACGTGTGA 3'

Probe S Univ-1390, a universal probe for any microorganism, with sequence SEQ ID [n°3] No. 3: 5' GACGGGCGGTGTGTACAA 3', and

Probe S Bac338, specific for bacteria, with sequence SEQ ID [n°4] No. 4: 5' GCTGCCTCCCGTAGGAGT 3'.

Pages 18 and 19, titled LIST OF SEQUENCES should read as follows:

LIST OF SEQUENCES

(1) General information:

(i) Applicant:
- (a) Name: Suez Lyonnaise des Eaux
- (b) Address: 72 avenue de la Liberté
- (c) City: Nanterre Cedex
- (e) Country: France
- (f) Postal code: 92753

(ii) Title of the invention: Means for qualitative and quantitative analysis of the microbial populations potentially present in a sample

(iii) Number of sequences: 4

(iv) Form readable by computer:

- (a) type of storage medium: floppy disk
- (b) computer: IBM PC compatible
- (c) operating system: PC-DOS/MS-DOS
- (d) software: PatentIn Release #1.0, Version #1.30 (OEB)

(2) Information for SEQ ID [n°1] No. 1:

(i) Characteristics of the sequence:

(a) Length: 15 base pairs
(b) Type: nucleotide
(c) Number of strands: single
(d) Configuration: linear

(ii) Type of molecule: other nucleic acid
(iii) Hypothetical: yes
(iv) Antisense: no
(vii) Immediate source:
(B) Clone: Nb1000

(xi) Description of the sequence: SEQ ID [n°1] No. 1:
TGCGACCGGT CATGG

(3) Information for SEQ ID [n°2] No. 2:

(i) Characteristics of the sequence:
(a) Length: 20 base pairs
(b) Type: nucleotide
(c) Number of strands: single
(d) Configuration: linear

(ii) Type of molecule: other nucleic acid
(iii) Hypothetical: yes
(iv) Antisense: no
(vii) Immediate source:
(B) Clone: Nb1225

(xi) Description of the sequence: SEQ ID [n°2] No. 2:
5' CGCCATTGTA TTACGTGTGA 3'

(4) Information for SEQ ID [n°3] No. 3:

(i) Characteristics of the sequence:
(a) Length: 18 base pairs
(b) Type: nucleotide
(c) Number of strands: single
(d) Configuration: linear

(ii) Type of molecule: other nucleic acid
(iii) Hypothetical: yes
(iv) Antisense: no
(vii) Immediate source:
(B) Clone: S Univ-1390

(xi) Description of the sequence: SEQ ID [n°3] No. 3:
5' GACGGGCGGTGTGTACAA 3'

(5) Information for SEQ ID [n°4] No. 4:

(i) Characteristics of the sequence:
(a) Length: 18 base pairs
(b) Type: nucleotide
(c) Number of strands: single
(d) Configuration: linear

(ii) Type of molecule: other nucleic acid
(iii) Hypothetical: yes
(iv) Antisense: no
(vii) Immediate source:
(B) Clone: S Bac338

(xi) Description of the sequence: SEQ ID [n°4] No. 4:
5' GCTGCCTCCCGTAGGAGT 3'

In the Claims

Please amend the following claims as follows:

1. (Thrice Amended) A method of qualitative and quantitative analysis of microbial population(s) comprising:

providing a sample containing miocroorganisms,

contacting the microorganisms present in [a] the sample with at least one specific probe to form a probe-target complex using *in situ* hybridization in whole cells, wherein the specific probe recognizes a RNA target sequence [under conditions favorable to *in situ* hybridization in whole cell],

extracting the hybridized specific probes from [their target] the probe-target complex in the contacted sample by adding a denaturing agent to denature the probe-target complex, and

detecting the extracted probes and measuring the amount thereof or their respective amounts to provide the qualitative and quantitative analysis of the microorganisms in the sample.

8. (Twice Amended) A method according to Claim 7, wherein fixation of the cells is achieved by incubation of the cells in a fixation solution of less than 10% paraformaldehyde for 3 to 12 hours at 4°C.

17. (Thrice Amended) A method according to Claim 1, wherein said detecting and measuring the amount [measurement] of the extracted probes includes detection and [amount measurement] quantification of a label associated with or incorporated into the extracted probes, wherein the label is a radioactive, chemiluminescent or fluorescent label.

19. (Thrice Amended) A method according to Claim 1, wherein [it] said method is used in combination with a process for triggering an alarm in connection with quality, safety and or sanitary monitoring of the product from which said sample has been obtained.